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SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to *Lactobacillus rhamnosus* GG and maintenance of normal defecation during antibiotic treatment pursuant to Article 13(5) of Regulation (EC) No 1924/2006¹

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following an application from Fuko Pharma Ltd, submitted pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Finland, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to *Lactobacillus rhamnosus* GG and maintenance of normal defecation during antibiotic treatment. The food constituent, *Lactobacillus rhamnosus* GG, which is the subject of the health claim, is sufficiently characterised. The claimed effect proposed by the applicant is “help to maintain normal defecation during antibiotic treatment” and the target population proposed by the applicant is “healthy outpatient adults and children on oral antibiotic treatment”. Maintenance of normal defecation during antibiotic treatment is a beneficial physiological effect. The Panel notes that the information submitted from five out of seven human intervention studies is insufficient to allow a full scientific evaluation, and that these studies have important methodological limitations. No conclusions could be drawn from these studies for the scientific substantiation of the claim. The remaining two human intervention studies, from which conclusions could be drawn for the scientific substantiation of the claim, did not show an effect of *Lactobacillus rhamnosus* GG on the incidence of diarrhoea resulting from antibiotic treatment. The Panel concludes that a cause and effect relationship has not been established between the consumption of *Lactobacillus rhamnosus* GG and maintenance of normal defecation during antibiotic treatment.

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KEY WORDS

Lactobacillus rhamnosus GG, defecation, antibiotic treatment, health claims

¹ On request from the Competent Authority of Finland following an application by Fuko Pharma Ltd, Question No EFSA-Q-2013-00015, adopted on 30 May 2013.

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SUMMARY

Following an application from Fuko Pharma Ltd, submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Finland, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to *Lactobacillus rhamnosus* GG and maintenance of normal defecation during antibiotic treatment.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.

The Panel considers that the food constituent, *Lactobacillus rhamnosus* GG, which is the subject of the health claim, is sufficiently characterised.

The claimed effect proposed by the applicant is “help to maintain normal defecation during antibiotic treatment”. The target population proposed by the applicant is “healthy outpatient adults and children on oral antibiotic treatment”. In the context of the studies provided for the scientific substantiation of this claim, the Panel notes that the target population is adults and children under antibiotic treatment. The Panel considers that maintenance of normal defecation during antibiotic treatment is a beneficial physiological effect.

The applicant identified seven human intervention studies and four meta-analyses of human intervention studies as being pertinent to the health claim.

Three out of the four intervention studies, which were conducted in adults, investigated the effect of *L. rhamnosus* GG on the occurrence of antibiotic-associated side-effects including diarrhoea in asymptomatic patients under *Helicobacter pylori* eradication therapy. Upon a request by EFSA for clarification, the applicant indicated that diarrhoea was defined as “persistent (at least three days) increased frequency or decreased consistency of bowel movements with respect to baseline” as assessed by the study subjects. The Panel notes that the information submitted for these three studies, both in the application and following a request for clarification by EFSA, was insufficient (e.g. imprecise criteria for self-diagnosed diarrhoea episodes, insufficient description of the statistical analyses, and insufficient description of the study population) to allow a full scientific evaluation. In addition, the three studies have important methodological limitations (e.g. multiple comparisons were not considered in the data analysis, and open label design for one study based on self-reported outcomes). The Panel considers that no conclusions can be drawn from these studies for the scientific substantiation of the claim.

The fourth intervention study, which was conducted in adults under erythromycin therapy, investigated the effect of *L. rhamnosus* GG on several symptoms including diarrhoea. The Panel notes that the information submitted for this study, both in the application and following a request for clarification by EFSA, is insufficient (e.g. insufficient description of the study population, no information on the validation of the procedure used for reporting symptoms, and results regarding the incidence of diarrhoea and the number of defecations per day were not reported) to allow a full scientific evaluation, and that this study has a high risk of bias due to important methodological limitations (e.g. no definition of diarrhoea, and inappropriate statistical analysis for the study design). The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

One out of three double-blind, placebo-controlled intervention studies, which were conducted in children, investigated the incidence of diarrhoea in 167 children who received capsules containing *L. rhamnosus* GG or placebo during an oral antibiotic treatment for acute respiratory infections. The primary outcome was the occurrence of diarrhoea, which was defined as at least three watery or loose stools per day for a minimum of two consecutive days, during the first two weeks after the beginning

of the antibiotic treatment. The Panel notes that results for the intention-to-treat analysis were not provided, and that this study reported a borderline significant effect of *L. rhamnosus* GG on the incidence of diarrhoea within two weeks of antibiotic therapy in the population of completers only. The Panel also notes the high dropout rate, and the lack of information on the number of subjects who dropped out and experienced diarrhoea. The Panel considers that this study does not show an effect of *L. rhamnosus* GG on the incidence of diarrhoea in children during antibiotic treatment.

In a single centre, double-blind, placebo-controlled study, 83 hospitalised *H. pylori*-positive children were randomised to receive a 7-day *H. pylori* eradication therapy plus capsules containing *L. rhamnosus* GG or placebo during the duration of the *H. pylori* eradication therapy. The primary outcome of the study was the rate of *H. pylori* eradication. The secondary outcomes were the proportion of patients with treatment-associated diarrhoea, defined as three or more loose or watery stools per day for a minimum of 48 hours occurring during and/or up to two weeks after the end of the antibiotic therapy; and other gastrointestinal side effects during *H. pylori* eradication therapy. The risk of treatment-associated diarrhoea was not significantly different between groups. The Panel notes that this study does not show an effect of *L. rhamnosus* GG on the incidence of diarrhoea during antibiotic treatment for *H. pylori* eradication, that the dose of *L. rhamnosus* GG used in the study was one logarithmic unit lower than that proposed in the conditions of use for this claim, and that power calculations were not performed.

A randomised, double-blind, placebo-controlled study investigated the effect of *L. rhamnosus* GG on the occurrence of diarrhoea in children on antibiotic treatment for acute infection of the upper or lower respiratory tract, urinary tract, soft tissues, or skin. Upon a request by EFSA for clarification, the applicant indicated that diarrhoea was defined as the presence of at least two liquid stools per day on at least two “observation periods” during the course of the study. The Panel notes that the information submitted in relation to this study, both in the application and following a request for clarification by EFSA, is insufficient (e.g. unclear description of the methods used for the statistical analysis of the data, poor data reporting, and imprecise definition of diarrhoea episodes) to allow a full scientific evaluation, and that the study has a high risk of bias due to important methodological limitations (e.g. the use of “observation periods” to define the presence of diarrhoea for data analysis was not scientifically justified). The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

The Panel considers that the meta-analyses provided by the applicant cannot be used for the scientific substantiation of the claim as they included only two studies, from one of which no conclusions could be drawn for the scientific substantiation of the claim on *L. rhamnosus* GG, or they included studies conducted with different strains of *L. rhamnosus* rather than with *L. rhamnosus* GG.

In weighing the evidence, the Panel considers that the only two human intervention studies from which conclusions could be drawn did not show an effect of *Lactobacillus rhamnosus* GG on the incidence of diarrhoea resulting from antibiotic treatment.

The Panel concludes that a cause and effect relationship has not been established between the consumption of *Lactobacillus rhamnosus* GG and maintenance of normal defecation during antibiotic treatment.

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BACKGROUND

Regulation (EC) No 1924/2006⁴ harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children's development and health) which are based on newly developed scientific evidence, or which include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Article 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA

- The application was received on 21/12/2012.
- The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.
- The scientific evaluation procedure started on 28/01/2013.
- On 28/02/2013, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The clock was stopped on 06/03/2013 and restarted on 21/03/2013, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
- On 26/03/2013, EFSA received the requested information (which was made available to EFSA in electronic format on 20/03/2013).
- During its meeting on 30/05/2013, the NDA Panel, having evaluated the data submitted, adopted an opinion on the scientific substantiation of a health claim related to *Lactobacillus rhamnosus* GG and maintenance of normal defecation during antibiotic treatment.

TERMS OF REFERENCE

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: *Lactobacillus rhamnosus* GG and maintenance of normal defecation during antibiotic treatment.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of *Lactobacillus rhamnosus* GG, a positive assessment of its safety, nor a decision on whether *Lactobacillus rhamnosus* GG is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

⁴ Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address: Fuko Pharma Ltd, Mikrokatu 1, PO Box 1188, 70211 Kuopio, Finland

Food/constituent as stated by the applicant

According to the applicant, the food constituent that is the subject of the health claim is *Lactobacillus rhamnosus* GG (ATCC n. 53103).

Health relationship as claimed by the applicant

According to the applicant, the health claim refers to the ability of *Lactobacillus rhamnosus* GG (ATCC n. 53103) to help to maintain normal defecation during antibiotic treatment. The applicant states that the mechanisms of action can result from different factors related to gut microbial ecology and interaction between the bacteria and host: the microbes abilities to digest food and compete for nutrients with pathogens, to alter local pH to create an unfavourable local environment for pathogens, to produce bacteriocins to inhibit or kill pathogens, to scavenge superoxide radicals, to down-regulate the expression of virulence factors required for pathogenesis, to enhance the immunity by interacting with the immune system, to stimulate epithelial mucin production, to enhance intestinal barrier function, to compete for adhesion with pathogens and/or their abilities to modify pathogen-derived toxins.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: "*Lactobacillus rhamnosus* GG for maintaining normal defecation during oral antibiotic treatment".

Specific conditions of use as proposed by the applicant

The applicant has proposed a daily dose of $2-4 \times 10^{10}$ CFU *Lactobacillus rhamnosus* GG (ATCC n. 53103) to be consumed as 2-4 capsules per day, divided into two doses per day, during oral antibiotic treatment. The target population is healthy outpatient adults and children on oral antibiotic treatment.

ASSESSMENT

1. Characterisation of the food/constituent

The food constituent that is the subject of the health claim is *Lactobacillus rhamnosus* GG.

The strain *L. rhamnosus* GG has been identified and characterised at species and strain level using both phenotypic and genotypic methods. The Panel notes that the culture collection number from the American Type Culture Collection (ATCC 53103) is given. The genome sequence of *L. rhamnosus* GG has been published by Kankainen et al. (2009).

Data on the manufacturing process, formulation into capsules, safety and stability were provided.

The Panel considers that the food constituent, *Lactobacillus rhamnosus* GG, which is the subject of the health claim, is sufficiently characterised.

2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is “help to maintain normal defecation during antibiotic treatment”. The target population proposed by the applicant is “healthy outpatient adults and children on oral antibiotic treatment”. In the context of the studies provided for the scientific substantiation of this claim, the Panel notes that the target population is adults and children under antibiotic treatment.

The outcome measures proposed by the applicant to assess the claimed effect include absence of diarrhoea, defined as decreased stool consistency and increased stool frequency, duration of diarrhoea, and absence of constipation.

Following a request by EFSA, the applicant clarified that the claimed effect relates to a reduction in the incidence of diarrhoea episodes during antibiotic treatment in subjects without diarrhoea at recruitment.

The Panel considers that maintenance of normal defecation during antibiotic treatment is a beneficial physiological effect.

3. Scientific substantiation of the claimed effect

The applicant performed a literature search in PubMed/Medline and in the Cochrane Database of Systematic Reviews in May 2012 using the search terms “*Lactobacillus* GG AND antibiotic”, “*Lactobacillus* GG” and “antibiotic-associated-diarrhea AND probiotics”, in order to identify studies on the effects of *L. rhamnosus* GG on defecation during antibiotic treatment. Studies with defecation as an outcome but unrelated to antibiotic treatment were excluded.

Based on the search criteria described above, the applicant identified seven human intervention studies (Siitonen et al., 1990; Arvola et al., 1999; Vanderhoof et al., 1999; Armuzzi et al., 2001a, b; Cremonini et al., 2002; Szajewska et al., 2009) and four meta-analyses of human intervention studies (Szajewska et al., 2006; Johnston et al., 2006, 2007, 2011) as being pertinent to the health claim.

The Panel notes that four of the human intervention studies (Armuzzi et al., 2001a, b; Cremonini et al., 2002; Szajewska et al. 2009) were conducted in asymptomatic patients undergoing eradication therapy, consisting of antibiotics plus proton pump inhibitors (PPI), for *H. pylori* infection. The Panel notes that diarrhoea and constipation are possible side effects of PPI. However, given that the incidence of these side effects in relation to treatment with PPI is comparable, and that such effects of PPI may have been equally distributed among study groups, the Panel considers that results from studies in patients undergoing eradication therapy for *H. pylori* can be used for the scientific substantiation of a claim on maintenance of normal defecation during antibiotic treatment.

The Panel also notes that, whereas in the studies by Szajewska et al. (2009) and by Vanderhoof et al. (1999) consumption of food products containing live microorganisms other than the study products was not allowed to minimise confounding, information on background consumption of live microorganisms during the intervention is lacking in the remaining studies.

Human intervention studies in adults

Four human intervention studies on the effects of *L. rhamnosus* GG during antibiotic treatment were conducted in adults (Siitonen et al., 1990; Armuzzi et al., 2001a, b; Cremonini et al., 2002).

In the double-blind, placebo-controlled study by Armuzzi et al. (2001a), 60 *H. pylori*-positive asymptomatic (i.e. absence of gastrointestinal symptoms) volunteers (male/female: 25/35; age 28-52 years) were recruited among the hospital staff and were randomised to receive a 7-day *H. pylori* eradication therapy (rabeprazole, clarithromycin, tinidazole) plus a freeze-dried powder containing *L. rhamnosus* GG (12×10^9 CFU/day) (n = 30) or an identical placebo without *L. rhamnosus* GG

(n = 30) for 14 days (i.e. during and the week after eradication therapy). At the end of the eradication therapy week and the last day of the three subsequent weeks, each subject reported the presence of symptoms (taste disturbance, loss of appetite, nausea, vomiting, stomach pain, bloating, diarrhoea, constipation, skin rash) and the severity of each symptom (mild, moderate, severe) through the self-reported questionnaire which was designed for evaluating *H. pylori* eradication therapy side-effects (de Boer et al., 1996), and which was modified to include bloating and constipation, as clarified by the applicant upon a request by EFSA. Subjects also provided an overall judgment of tolerability based on a five point scale. For each symptom, the relative risk (RR) and 95 % confidence interval (CI) was calculated.

In an open label study by the same research group (Armuzzi et al., 2001b), 120 *H. pylori*-positive asymptomatic (i.e. absence of dyspeptic symptoms) volunteers (male/female: 54/66; age 26-48 years) were recruited among hospital staff and randomised to receive a 7-day *H. pylori* eradication therapy (pantoprazole, clarithromycin, tinidazole) with or without a freeze-dried powder containing *L. rhamnosus* GG. This study investigated the same outcomes as the study by Armuzzi et al. (2001a) and at the same time points. The Panel notes that open label studies, as in this study, have a high risk of bias for self-reported outcomes.

In a double-blind, placebo-controlled study by the same research group (Cremonini et al., 2002), a total of 97 *H. pylori*-positive asymptomatic (i.e. absence of gastrointestinal symptoms) volunteers (male/female: 43/54; age 18-61 years) were enrolled. Subjects were excluded if they had any symptoms or consumed any “drug associated with gastro-intestinal side effects” during the three week run-in period. After the run-in period, 85 subjects under a 7-day *H. pylori* eradication therapy (rabeprazole, clarythromicin, tinidazole) were randomised to one of the following four arms: a freeze-dried powder containing *L. rhamnosus* GG (12×10^9 CFU/day) (group I; n = 21), or a *Saccharomyces boulardii* preparation (10×10^9 CFU/day) (group II; n = 22), or a combination of *Lactobacillus acidophilus* and *Bifidobacterium lactis* (10×10^9 CFU/day) (group III; n = 21), or placebo (identical pack as for the treatments) (group IV; n = 21). The study products were consumed twice a day during the week of the *H. pylori* eradication therapy and the week afterwards. This study investigated the same outcomes as the studies by Armuzzi et al. (2001a, b) and at the same time points. The authors claimed that a total sample size of 73 subjects was calculated as appropriate to detect a difference of 20 % in symptom occurrence between active treatment and placebo groups, assuming an expected incidence of any side effect in 25 % of subjects treated with antibiotics, with an 80 % power to detect differences and a two-sided α of 0.05. Considering the four intervention arms in this study, it is unclear to the Panel how the total sample of 73 subjects needed was calculated.

The Panel noted that in the studies by Armuzzi et al. (2001a, b) and by Cremonini et al. (2002), diarrhoea episodes were not defined in the publications nor in the questionnaire by Boer et al. (1996), that the primary outcome was not identified and power calculations were not performed except for the study by Cremonini et al. (2002), that multiple comparisons were not taken into account in the data analyses, and that baseline characteristics of the subjects recruited were not reported. Upon a request by EFSA for clarification on these points, the applicant indicated that diarrhoea was defined as “persistent (at least three days) increased frequency or decreased consistency of bowel movements with respect to baseline” as assessed by the study subjects, that the primary outcome of these studies was the occurrence of antibiotic-associated side-effects, that no power calculations were performed for two of the studies (Armuzzi et al., 2001a, b), that adjustments for multiple comparisons were not considered, and that, although the applicant stated that no significant differences were observed between intervention and control groups in any of the studies at baseline, baseline characteristics of subjects were not available for these studies.

The Panel notes that the additional information submitted in relation to these studies (Armuzzi et al., 2001a, b; Cremonini et al., 2002) upon a request by EFSA is insufficient to allow a full scientific evaluation (e.g. imprecise criteria for self-diagnosed diarrhoea episodes, insufficient description of the statistical analyses, and insufficient description of the study population). In addition, the Panel notes

that these studies have important methodological limitations (e.g. multiple comparisons were not considered in the data analysis, and open label design for one study based on self-reported outcomes (Armuzzi et al., 2001b)). The Panel considers that no conclusions can be drawn from these studies for the scientific substantiation of the claim.

In the study by Siitonen et al. (1990), 16 male subjects (age 18-24 years) were randomly assigned to receive erythromycin acistrate plus a yogurt fermented with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* and pasteurised afterwards, and then either supplemented with *L. rhamnosus* GG (5×10^9 CFU/day) (n = 8) or non-supplemented (placebo; n = 8) for one week. Participants were invited to report symptoms such as diarrhoea, stomach pain and nausea, as well as the number of defecations per day and faecal volume, by completing daily records and during an interview with a physician before and after the study. The results of observations on the side effects were analysed statistically using the Chi-square and student's t-tests. The Panel notes that baseline characteristics of the study population were not reported, that no power calculations were performed, that no information on the validation of the procedure used for reporting symptoms was provided, that diarrhoea was not defined, that results regarding the incidence of diarrhoea and the number of defecations per day were not reported, and that the statistical analysis is inappropriate for the study design (e.g. multiple comparisons were not considered). Following a request for clarification by EFSA, no further information was provided. The Panel notes that the information submitted in relation to this study is insufficient (e.g. insufficient description of the study population, no information on the validation of the procedure used for reporting symptoms, and results regarding the incidence of diarrhoea and the number of defecations per day were not reported) to allow a full scientific evaluation and that the study has a high risk of bias due to important methodological limitations (e.g. no definition of diarrhoea, and inappropriate statistical analysis for the study design). The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

Human intervention studies in children

Three human intervention studies on the effects of *L. rhamnosus* GG during antibiotic treatment were conducted in children (Arvola et al., 1999; Vanderhoof et al., 1999; Szajewska et al., 2009).

In a randomised, double-blind, placebo-controlled study, Arvola et al. (1999) investigated the incidence of diarrhoea in 167 children who received capsules containing *L. rhamnosus* GG (4×10^{10} CFU/day) (n = 89) or placebo (microcrystalline cellulose) (n = 78) during an oral antibiotic treatment (7-10 days) for acute respiratory infections. Exclusion criteria were antibiotic treatment during the previous three months, gastrointestinal disorders, and need for intravenous antibiotic treatment. All subjects except five (hospitalised) were outpatients. The primary outcome was diarrhoea during the first two weeks after the beginning of the antibiotic treatment. Diarrhoea was defined as at least three watery or loose stools per day for a minimum of two consecutive days. Parents kept daily symptom diaries and recorded stool frequency and consistency (solid, loose, watery) at home for three months; with respect to diarrhoea, faecal samples were taken for viral and bacterial analyses. Secondary outcomes were the activities of faecal urease, β -glucuronidase, and β -glucosidase. A total of 28 (31 %) and 20 (26 %) subjects from the *L. rhamnosus* GG and placebo groups, respectively, were lost at follow-up or discontinued the study; one reason, among other unspecified reasons given, was the difficulty in the transportation of the study samples. No information on the number of subjects who dropped out and experienced diarrhoea in the intervention and in the control groups was provided in the application or following a request for clarification by EFSA. Data analyses were undertaken in the sample of completers only (n = 119; mean age 4.5 years; age range from two weeks to 12.8 years; 72 % < 6 years), who were comparable at baseline regarding mean age, clinical diagnosis, antibiotics, history of antibiotic use, and mode of day care. Intention-to-treat (ITT) analyses were not reported in the publication and were not provided upon a request by EFSA. In the population of completers, the incidence of diarrhoea within two weeks of antibiotic therapy was 5 % (3/61) in the *L. rhamnosus* GG group and 16 % (9/58) in the placebo group

($X_2 = 3.82$; $p = 0.05$). The treatment effect (95 % CI) of *L. rhamnosus* GG was -11 % (-21 %-0 %). The severity of diarrhoea (mean stool frequency five times per day; range: 3-6) and the duration of diarrhoea (mean 4 days; range: 2-8) were not different between groups. In diarrhoeal episodes, the viral and bacterial analyses were positive for *Clostridium difficile* in two cases (one in both groups) and Norwalk-like calicivirus in three cases (one in the intervention group and two in the placebo group). The Panel notes that this study reported a borderline significant effect of *L. rhamnosus* GG on the incidence of diarrhoea during antibiotic treatment in the population of completers only. However, the Panel also notes the high dropout rate, the lack of information on the number of subjects who dropped out and experienced diarrhoea, and that results for the ITT analysis were not provided. The Panel considers that this study does not show an effect of *L. rhamnosus* GG on the incidence of diarrhoea in children during antibiotic treatment.

In the single centre, double-blind, placebo-controlled study by Szajewska et al. (2009), 83 hospitalised *H. pylori*-positive children (male/female: 39/44; age 5-17 years) were randomised to receive a 7-day *H. pylori* eradication therapy (amoxicillin, clarithromycin, omeprazole) plus capsules containing *L. rhamnosus* GG (2×10^9 CFU/day) ($n = 44$) or placebo (maltodextrine) ($n = 39$) during the duration of the *H. pylori* eradication therapy. *H. pylori* infection was confirmed by two of the three following tests: the ^{13}C -urea breath test (UBT), histopathology (haematoxylin and eosin staining), the rapid urease test. Exclusion criteria were acute or chronic gastrointestinal disease, current use of antacids, and use of antibiotics in the previous seven days. The primary outcome of the study was the rate of *H. pylori* eradication, which had to be confirmed by a negative UBT at least four weeks after treatment. The secondary outcomes were the proportion of patients with treatment-associated diarrhoea, defined as three or more loose or watery stools per day for a minimum of 48 hours occurring during and/or up to two weeks after the end of the antibiotic therapy; any gastrointestinal side effects, including abdominal pain, nausea, vomiting, constipation, flatulence, taste disturbance, or loss of appetite, during *H. pylori* eradication therapy; the need for discontinuation of the *H. pylori* eradication therapy. Each patient received forms/diaries to record the frequency of side effects and any symptoms they considered important; the forms/diaries were completed at study entry, at day 7 (end of treatment), at day 21 (two weeks after treatment), and at four to six weeks after treatment. The children were evaluated clinically at study entry, at day 7, at day 21, and at 4 to 6 weeks after enrolment. A total of 17 children (10 in the treatment group and 7 in the placebo group) were excluded from the analysis because of lack of diaries and/or UBT at follow-up. The Panel notes that exclusion of children with no UBT at follow-up is not appropriate for the analysis of the outcome measure (risk of antibiotic-associated diarrhoea) of interest in relation to this claim. The risk of antibiotic-associated diarrhoea was not significantly different between groups (*L. rhamnosus* GG group 2/34 (6 %) vs placebo group 6/30 (20 %), $\text{RR} = 0.3$, 95 % CI 0.07–1.2). The Panel notes that this study does not show an effect of *L. rhamnosus* GG on the incidence of diarrhoea during antibiotic treatment for *H. pylori* eradication in children. The Panel also notes that the dose of *L. rhamnosus* GG used in the study was one logarithmic unit lower than that proposed in the conditions of use for this claim, and that power calculations were not performed.

In a randomised, double-blind, placebo-controlled study (Vanderhoof et al., 1999), 202 children (age from 6 months to 10 years) recruited from a private primary care paediatric practice on a 10-day antibiotic treatment for acute infection of the upper or lower respiratory tract, urinary tract, soft tissues, or skin were randomised to receive capsules containing inulin (325 mg) plus *L. rhamnosus* GG (1×10^{10} CFU/day if weight < 12 kg or 2×10^{10} CFU/day if weight > 12 kg) ($n = 100$) or placebo (inulin, 325 mg; $n = 102$).

It was reported that 202 children were randomised based on power calculations ($\alpha = 0.05$ and 0.80 power), but no information (e.g. primary outcome, target difference between groups, variability of the selected outcome) was provided on how these calculations were performed. Children with any chronic disease, serious acute infection, or diarrhoea at the time of antibiotic initiation were excluded from this study. Parents were contacted within 24 hours of initial enrolment for baseline data collection by one of the investigators, and subsequently every three days until antibiotic courses were

completed or diarrhoea ceased. Parents were asked to complete a questionnaire reporting on gastro-intestinal events. At each contact with the parents, stool frequency (number of stools passed during a 24-hour period) and consistency (based on eight line drawings depicting stools varying from watery to hard and dry) were assessed and graded numerically. Diarrhoea was defined as the presence of at least two liquid stools per day on at least two “observation periods” during the course of this study. Upon a request for clarification by EFSA, the applicant indicated that one “observation period” consisted of 3-4 days during the study period (i.e. days 1-4, days 4-7 and days 7-10 being the 1st, 2nd and 3rd observation periods, respectively), and that the definition of diarrhoea included that at least two liquid stools per day were observed at least in two of these periods, e.g. diarrhoea was continuous or was repeated at least twice. The Panel notes that diarrhoea episodes were imprecisely defined (i.e. the number of consecutive days with at least two liquid stools per day was not specified) and that the subdivision of the whole study period into “observation periods” consisting of three days each to define the presence of diarrhoea for data analysis was not scientifically justified. The presence or absence of visible blood content in the stool, abdominal pain, nausea, vomiting, bloating, and appetite suppression were also assessed. If present, the intensity of abdominal pain was recorded on a visual analog scale. The Panel notes that the primary outcome of the study was not identified in the publication, and that a large number of outcome measures were assessed.

A total of 14 children failed to complete the study because of antibiotic non-compliance or inability of the investigators to contact the parents at the assigned follow-up time. None of the participants failed to complete the 10-day course of antibiotics because of a change in stool consistency or frequency. Statistical analyses were carried out in the population of completers only. The Panel notes that reasons for dropping out are given. It was reported that stool consistency scores and stool frequency were analysed by a mixed design, groups observation point, analysis of variance, and that Newman-Keuls pairwise comparisons were performed. It was also reported that Chi-squared analysis was performed to evaluate the occurrence of the stool consistency score of < 4 on either day 7 or day 10 of the observation period. The Panel notes that the description of the methods used for the statistical analysis of the data is unclear and insufficient for a scientific evaluation. No further information, except for the p-values of a Chi-squared analysis in relation to the incidence of diarrhoea, was provided upon a request by EFSA.

The Panel notes that the additional information submitted in relation to this study in response to a request by EFSA is insufficient (e.g. unclear description of the methods used for the statistical analysis of the data, poor data reporting, and imprecise definition of diarrhoea episodes) to allow a full scientific evaluation, and that the study has a high risk of bias due to important methodological limitations (e.g. the use of “observation periods” to define the presence of diarrhoea for data analysis was not scientifically justified). The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

Meta-analyses of human intervention studies in children

The meta-analyses by Johnston et al. (2006) and Szajewska et al. (2006) included subgroup analyses of two studies using *L. rhamnosus* GG (Arvola et al., 1999; Vanderhoof et al., 1999) which have been described above. The Panel notes that subgroup analyses in both of these meta-analyses only included two studies, one of which was the study by Vanderhoof et al. (1999) which provided insufficient data for the scientific substantiation of the claim. The Panel considers that no conclusions can be drawn from these meta-analyses for the scientific substantiation of the claim.

The meta-analysis by Johnston et al. (2008) reported the results of an analysis on the effects of *L. rhamnosus* GG (studies by Arvola et al. (1999) and Vanderhoof et al. (1999)) combined with other strains of *Lactobacillus rhamnosus* on the incidence of diarrhoea during antibiotic use. The meta-analysis by Johnston et al. (2011) included the studies by Arvola et al. (1999), Szajewska et al. (2009) and Vanderhoof et al. (1999), which were considered pertinent by the applicant and have been described above, in addition to intervention studies using other strains and strain combinations. The

Panel considers that these meta-analyses which combine the results from studies conducted with different strains of *L. rhamnosus* cannot be used for the scientific substantiation of a claim on *L. rhamnosus* GG.

In weighing the evidence, the Panel considers that the two human intervention studies (Arvola et al., 1999; Szajewska et al., 2009) from which conclusions could be drawn for the scientific substantiation of the claim did not show an effect of *Lactobacillus rhamnosus* GG on the incidence of diarrhoea resulting from antibiotic treatment.

The Panel concludes that a cause and effect relationship has not been established between the consumption of *Lactobacillus rhamnosus* GG and maintenance of normal defecation during antibiotic treatment.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food constituent, *Lactobacillus rhamnosus* GG, which is the subject of the health claim, is sufficiently characterised.
- The claimed effect proposed by the applicant is “help to maintain normal defecation during antibiotic treatment”. The target population proposed by the applicant is “healthy outpatient adults and children on oral antibiotic treatment”. Maintenance of normal defecation during antibiotic treatment is a beneficial physiological effect.
- A cause and effect relationship has not been established between the consumption of *Lactobacillus rhamnosus* GG and maintenance of normal defecation during antibiotic treatment.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on *Lactobacillus rhamnosus* GG and maintenance of normal defecation during antibiotic treatment pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0372_FI). December 2012. Submitted by Fuko Pharma Ltd.

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GLOSSARY/ABBREVIATIONS

ATCC	American Type Culture Collection
CI	Confidence interval
CFU	Colony forming units
ITT	Intention-to-treat
PPI	Proton pump inhibitors
RR	Relative risk
UBT	¹³ C-urea breath test